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Aging: From Radiant Youth to an Abrupt End

Dispatch

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A gene that controls growth rate, radiation resistance and lifespan in the nematode Caenorhabditis elegans has been found to encode a homologue of a yeast telomere maintenance factor, raising the possibility that checkpoint control, telomere maintenance and aging may be linked in unanticipated ways.

"*In youth we run into difficulties. In old age difficulties run into us.*" Josh Billings

The difficulties we encounter in life make us feel older for good reason: cellular stresses accelerate aging and limit our lifespan. With the recognition that life is inevitably followed by a precisely invariant endpoint, we have pondered ways to deflect the inexorable progress of aging. Most aging researchers (and commentators on the subject) have already experienced the degenerative changes that progress with time, and quietly hope that a molecular understanding of aging will soon — very soon — lead to the development of longevity-promoting drugs. While aging is a broadly defined biological process, many of the molecular events of the aging process have been revealed in the past decade, particularly from the emergence of systems in which longevity can be manipulated genetically [1]. Among the influences on aging and mortality, such processes as metabolic rate, oxidative damage, DNA repair and checkpoint control, gene silencing and telomere maintenance have received particular attention. A startling outcome from these discoveries is that senescence of unicellular yeast cells and aging in multicellular animals are influenced by some of the same molecular control systems [1].

Two processes that affect aging are mechanistically intertwined: several proteins involved in DNA checkpoint control, which causes cell-cycle arrest or apoptosis in response to DNA damage, also function in maintenance of telomeres, the specialized ends of linear chromosomes [2]. For example, the yeast protein Tel1, required for normal telomere length, is the homologue of human ataxia telangiectasia kinase, a mediator of DNA damage checkpoint control. Moreover, resistance to radiation in the budding yeast *Saccharomyces cerevisiae* and the nematode *Caenorhabditis elegans* requires a protein — Rad17 in yeast, Mrt-2 in worms — which activates checkpoint control [3]. Rad17/Mrt-2 also functions in telomere maintenance: *C. elegans* cells deficient for this protein show progressive telomere shortening and chromosome fusion [4]. Given that improper telomere maintenance can cause cells to senesce, and that increased telomerase activity can immortalize some cells (reviewed in [5]), it is not surprising that lesions in this gene also cause late-onset sterility. Cells of the germline — an immortal lineage in all non-extinct species — lose their immortality in telomerase mutants, concomitant with progressive telomere shortening and ultimately senescence [4].

Recent findings by three groups [6–8] further underscore the potential link between telomere maintenance, checkpoint control and longevity, but in rather unanticipated ways (Figure 1). *C. elegans clk-2* is one of four genes identified on the basis of their 'slow' mutant phenotype: *clk* mutants show delayed development, slower cell division rates and slowed neuronal cycles [9]. They are also longevity mutants, living up to 30% longer than wild-type worms. An allele of *clk-2*, called *rad-5*, was identified many years earlier in a separate screen for radiation-sensitive mutations [10], and was later found to abrogate radiation-induced checkpoint control — both cell-cycle arrest and apoptosis — in the germline [3]. Finally, *clk-2* was also picked out in a screen through existing longevity mutants for those with abnormal telomere lengths [8]. Thus it was that three separate groups [6–8], motivated by the distinct problems of aging/biological timing, checkpoint control and telomere function, respectively, all cloned *clk-2*/*rad-5* and found that it encodes a homologue of Tel2, a protein required for normal telomere length in yeast.

Tel2 was identified in screens for yeast mutations that result in abnormally short telomeres [11]. The telomeres of *tel2* mutant yeast cells are stably much shorter than normal telomeres. Like *clk-2* mutants of *C. elegans*, viable *tel2* mutant yeast cells grow slowly. Given the defining phenotype for yeast *tel2* mutants, it is therefore not surprising that Lim *et al.* [8] found that a *clk-2* allele results in shortened telomeres. However, the story is not so simple: Bénard *et al.* [6] found that telomeres were reproducibly *longer* in the *clk-2* mutants, that this effect was rescued by the wild-type gene, and that overexpression of the intact gene caused shortened telomeres. Finally, as though to ensure the longevity of the debate, Ahmed *et al.* [7] found no trend in either direction. The latter investigators explained the discrepancy by noting that extensive, apparently stochastic, heterogeneity in telomere length is seen between, and even within, individual strains of *C. elegans*.

Even if there is no effect of *clk-2* mutations on telomere length, this does not mean *a priori* that telomere maintenance defects are not responsible for the phenotypes of *clk-2* mutants. Telomere length was originally thought to be the critical parameter in telomere function that protects against senescence. For example, the replicative potential, or longevity, of dividing cells has been correlated with their ability to maintain telomeres at a sufficient length, a function requiring telomerase, which is necessary, and in some

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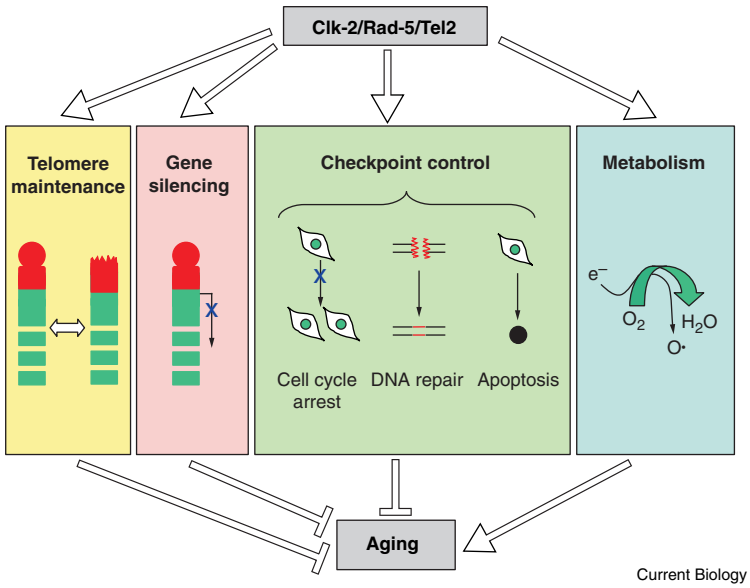

Figure 1. Pathways through which Clk-2/Rad-5 may affect aging.

The yeast protein Tel2 has been implicated in subtelomeric gene silencing and in telomere maintenance [5], although such a role in *C. elegans* is controversial. The worm protein is involved in genotoxic stress-induced checkpoint control and DNA repair; although existing mutations do not implicate the yeast protein in these processes, it does appear to function in chromosome stability [7], suggesting such a role. As the yeast and/or *C. elegans* protein is apparently required for these three mechanisms, which *inhibit* aging, only the last process, metabolism, a generator of oxygen radicals that accelerate aging, is readily understandable assuming the various groups [6–8] have studied simple loss-of-function mutations — all alleles of the *C. elegans* gene result in slow growth and behavior, and presumably attenuated metabolic rates. (I thank Adam Rothman for his assistance in preparing this figure.)

cases sufficient, for longevity of dividing cells [5]. However, it has since been shown that it is not the *length* of telomeres, but rather a stabilizing process, or capping, that is critical for proper telomere function and continued propagation of cells (reviewed in [5]). Clk-2/Rad-5 might function in telomere capping, which protects the double-strand ends of chromosomes from fusing to other such ends by the machinery that repairs double-strand DNA breaks. Consistent with such a notion, yeast Tel2 binds to telomere sequences [12], suggesting that its telomere-maintenance function might involve direct interaction with telomeres.

On the surface, then, one might imagine that Clk-2/Rad-5/Tel2 influences longevity by affecting the maintenance of telomeres in somatic nuclei. This seems unlikely, however. First, Bénard *et al.* [6] made the surprising observation that a Clk-2 fusion protein resides in the cytoplasm, not the nucleus as expected for a protein that acts by binding to telomeres. Of course, some of the protein might be nuclear but undetectable, or it may function indirectly in telomere maintenance. Even so, it is difficult to imagine how telomere maintenance might affect longevity of somatic tissues in an organism such as *C. elegans*, where all adult somatic cells arise by a limited and fixed number of cell divisions [13]. The longevity of yeast, vertebrate cells and the immortal *C. elegans* germline is defined by, or requires, continued mitosis of non-senescing cells, which necessitates continuous telomere maintenance [5]. In contrast, senescence of worms occurs long after cell division has ceased and longevity does not (and cannot) depend on replacement of somatic cells.

The longevity of *clk-2/rad-5* mutants may be attributable not to telomere maintenance but to another aging-related process — DNA repair. Clk-2/Rad-5 defines a new checkpoint component required for resistance to radiation-induced DNA damage [3,7]. However, this phenotype is paradoxical: DNA repair extends lifespan, and defects in it should diminish rather than extend lifespan. Indeed, that is exactly what is seen for mutations in other checkpoint genes, including mutations of the *p53* tumor suppressor, which reduce lifespan in both worms [14,15] and mice [16], and mutations in *mrt-2*, which extinguish the immortality of the *C. elegans* germline [4]. A complicating factor is that the *clk-2/rad-5/tel2* mutations are not null mutations in either yeast or worms, and the mutations studied might alter the protein in quite different ways, accounting for the discrepancy in phenotypes [6,7,11]. As elimination of the gene in either organism is lethal, its absolute roles in checkpoint control, telomere function and lifespan are not readily assessed.

DNA checkpoint control and telomere maintenance are linked in another way which could account for the longevity of *clk-2* mutants: some proteins involved in both processes also act in silencing of telomere-proximal genes [1]. Normally silent subtelomeric genes are desilenced in aging yeast, resulting in their sterility, and silencing of ribosomal (r)DNA leads to increased lifespan. This silencing is mediated by Sir proteins, which are telomere-associated in young yeast cells and move to the nucleolus in old cells. Remarkably, increasing the dosage of Sir2, the NAD-dependent histone deacetylase activity of which is responsible for silencing, increases lifespan in both yeast and in worms [17]. Caloric restriction, which extends lifespan, may act in part by increasing NAD levels, thereby activating Sir2's gene silencing function [1]. A mutation in yeast *tel2* reduces subtelomeric gene silencing, thus linking *clk-2/rad-5/tel2* gene anda process that influences aging. Again, however, the *clk-2* mutant phenotype is the opposite of what might be expected, as desilencing in yeast is associated with *decreased* longevity. Perhaps many of the *rad-5/clk-2* phenotypes in worms arise from desilencing of telomere-proximal genes, including genes that slow neuronal cycles or enhance longevity.

Such a mechanism could explain how a telomere maintenance function might regulate longevity of non-dividing somatic cells in *C. elegans*.

Given their slowed growth and development, *clk-2* mutants may be metabolically depressed, a condition that increases longevity irrespective of any effects on checkpoint control or telomere maintenance. Indeed, *clk-2* mutant adults are generally sickly and sluggish, suggesting reduced metabolic rates [6]. In fact, although *clk-2/rad-5* is the only *clk* gene known to affect checkpoint control [7], and the molecular identities of the *clk-1–clk-3* gene products indicate that they are involved in quite different molecular processes, mutations in ten *clk* genes have been identified based on slow growth phenotypes, and all extend lifespan [18]. Moreover, worms are no exception in showing longer lifespan as a result of caloric restriction: for example, semi-starved '*eat*' mutants show extended lifespan and appear to be defective in the same aging pathway as *clk-1* [19]. This does not, however, explain why checkpoint control and/or telomere maintenance defects lead to slow growth. Perhaps slow growth results from an unidentified checkpoint function that attenuates cell-cycle progression in response to damage accumulating in the absence of the Rad-5/Clk-2-mediated checkpoint. The slow neuronal cycles might be explained if damaged differentiated cells have mechanisms to reduce metabolic rates.

A major regulatory process for aging in *C. elegans* is an insulin-like hormonal signaling system that limits lifespan [1]. This pathway is apparently separate from that affected by the caloric restriction/*clk* pathway. But while the effect of the hormonal pathway on longevity may be separable from its role in metabolic regulation, it does appear to affect longevity through the oxidative stress which arises from electron transport [20]. Thus, once again the aging mechanism relates back to stress-management systems and metabolism. Teasing apart the function of factors that influence the complex process of aging and perform multiple activities within cells poses great challenges. Which of the activities are truly aging-relevant? Do *clk-2/rad-5* mutations extend longevity by affecting telomere maintenance, gene silencing, altered DNA repair/checkpoint control or simply by slowing growth and metabolism (Figure 1)? The degree to which the controls over DNA damage checkpoints, the abrupt ends of chromosomes and the finite span of life are intimately intertwined will be illuminated once the field reaches an advanced age. Many of us hope that will occur before we ourselves have arrived at that same stage.

References

1. Guarente, L., Kenyon, C.J. (2000). Genetic pathways that regulate ageing in model organisms. Nature *408*, 255–262.
2. Gasser, S.M. (2000). A sense of the end. Science *288*, 1377–1379.
3. Gartner, A. Milstein, S., Ahmed, S., Hodgkin, J. and Hengartner, M.O. (2000). A conserved checkpoint pathway mediates DNA damage-induced apoptosis and cell cycle arrest in *C. elegans.* Mol. Cell *5*, 435–443.
4. Ahmed, S. and Hodgkin, J. (2000). MRT-2 checkpoint protein is required for germline immortality and telomere replication in *C. elegans*. Nature *403*, 159–164.
5. Blackburn, E.H. (2000). Telomere states and cell fates. Nature *408*, 53–56.
6. Bénard, C., McCright, B., Zhang, Y., Felkai, S., Lakowski, B. and Hekimi, S. (2001). The *C. elegans* maternal-effect gene clk-2 is essential for embryonic development, encodes a protein homologous to yeast Tel2p and affects telomere length. Development *128*, 4045–4055.
7. Ahmed, S., Alpi, A., Hengartner, M.O. and Gartner, A. (2001). C. elegans RAD-5/CLK-2 defines a new DNA damage checkpoint protein. Curr. Biol. *11*, 1934–1944.
8. Lim, C.-S., Mian, S., Dernburg, A.F. and Campisi, J. (2001). *C. elegans* clk-2, a gene that limits life span, encodes a regulator of telomere metabolism similar to the yeast telomere binding protein Tel2p. Curr. Biol. *11*, 1706–1710.
9. Lakowski, B. and Hekimi, S. (1996). Determination of life-span in *Caenorhabditis elegans* by four clock genes. Science *272*, 1010–1013.
10. Hartman, P.S. and Herman, R.K. (1982). Radiation-sensitive mutants of *Caenorhabditis elegans*. Genetics *102*, 159–178.
11. Runge, K.W. and Zakian, V.A. (1996). TEL2, an essential gene required for telomere length regulation and telomere position effect in Saccharomyces cerevisiae. Mol. Cell Biol. *16*, 3094–3105.
12. Kota, R.S. and Runge, K.W. (1998). The yeast telomere length regulator TEL2 encodes a protein that binds to telomeric DNA. Nucleic Acids Res. 26, 1528–1535.
13. Sulston, J.E., Schierenberg, E., White, J.G. and Thomson, J.N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. *100*, 64–119.
14. Derry, W.B., Putzke, A.P. and Rothman, J.H. (2001). *Caenorhabditis elegans* p53: role in apoptosis, meiosis, and stress resistance. Science *294*, 591–595.
15. Schumacher, B., Hofmann, K., Boulton, S. and Gartner, A. (2001). The *C. elegans* homolog of the p53 tumor suppressor is required for DNA damage-induced apoptosis. Curr. Biol. *11*, 1722–1727.
16. Tyner, S. D., Venkatachalam, S., Choi, J., Jones, S., Ghebranious, N., Igelmann, H., Lu, X., Soron, G., Cooper, B., Brayton, C. *et al.* (2002). p53 mutant mice that display early ageing-associated phenotypes. Nature *415*, 45–53.
17. Tissenbaum, H.A. and Guarente, L. (2001). Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. Nature *410*, 227–230.
18. Hekimi, S., Burgess, J., Bussière, F., Meng, Y, and Bénard, C. (2001). Genetics of lifespan in *C. elegans*: molecular diversity, physiological complexity, mechanistic simplicity. Trends Genet. *17*, 712-718.
19. Lakowski, B. and Hekimi, S. (1998). The genetics of caloric restriction in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. U.S.A. *95*, 13091–13096.
20. Feng, J., Bussière, F. and Hekimi, S. (2001). Mitochondrial electron transport is a key determinant of life span in *Caenorhabditis elegans*. Dev. Cell *1*, 633–644.